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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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## METHOD OF TREATING CHRONIC CARDIAC DISEASE

This application claims priority of U.S. provisional application Serial No. 60/116,736 filed January 22, 1999, the disclosure of which is incorporated herein by reference.

The present invention relates generally to novel therapeutic uses of BPI protein products that involve treatment of chronic cardiac disease including, but not limited to, chronic states such as congestive heart failure and cardiomyopathy.

### BACKGROUND OF THE INVENTION

Chronic cardiac disease is a leading cause of mortality and morbidity in the developed world. One type of chronic cardiac disease is cardiomyopathy, which is actually a diverse group of diseases characterized by myocardial dysfunction that is not related to the usual causes of heart disease such as coronary atherosclerosis, valvular dysfunction and hypertension. Cardiomyopathies are categorized hemodynamically into dilated, hypertrophic, restrictive and obliterative cardiomyopathy, and can be of known or idiopathic etiology. Among the etiologies of dilated cardiomyopathy are pregnancy, drugs and toxins, such as alcohol, cocaine and chemotherapeutic agents (including doxorubicin and daunorubicin, dactinomycin, dacarbazine, cyclophosphamide, mitomycin, and anthracycline), and infectious and autoimmune processes. Hypertrophic cardiomyopathy is hereditary in more than 50% of cases and has a distinctive pattern of myocardial hypertrophy (thickening of muscle) usually with a pattern of asymmetrical thickening of the interventricular septum (also called asymmetrical septal hypertrophy). Restrictive cardiomyopathies are usually the product of an infiltrative disease of the myocardium, such as amyloidosis, hemochromatosis or a glycogen storage disease, and may also be seen in certain diabetic patients. Obliterative cardiomyopathy can be caused by endomyocardial fibrosis and hypereosinophilic syndrome. A common complication of all of the cardiomyopathies is progressive congestive heart failure.

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Congestive heart failure is often defined as the inability of the heart to deliver a supply of oxygenated blood sufficient to meet the metabolic needs of peripheral tissues at normal filling pressures. Chronic congestive heart failure can result as a consequence of coronary artery disease, cardiomyopathy, myocarditis, aortic stenosis, hypertension, idiopathic asymmetrical septal hypertrophy, coarctation of the aorta, aortic regurgitation, mitral regurgitation, left-to-right shunts, hypertrophied muscle, pericardial tamponade, constrictive pericarditis, mitral stenosis, left atrial myxoma, left atrial thrombus, cor triatriatum and numerous other conditions. Congestive heart failure is generally distinguished from other causes of inadequate oxygen delivery, such as circulatory collapse from hemorrhage or other causes of severe volume loss, congestion caused by fluid overload and high-output failure caused by increased peripheral demands which occurs in conditions such as thyrotoxicosis, arteriovenous fistula, Paget's disease and anemia. Therapy for congestive heart failure typically focuses on the treating the underlying etiology and the symptoms of fluid overload and heart failure. Chronic congestive heart failure that persists after correction of reversible causes is treated with diuretics (including thiazides such as chlorothiazide and hydrochlorothiazide, loop diuretics such as ethacrynic acid, furosemide, torsemide and bumetanide, potassium sparing agents such as spironolactone, triamterene and amiloride, and others such as metolazone and other quinazoline-sulfonamides), vasodilators (including nitroglycerin, isosorbide dinitrate, hydralazine, sodium nitroprusside, prostacyclin, captopril, enalapril, lisinopril, quinapril and losartan), positive inotropic agents (such as digitalis or digoxin), occasionally beta blockers, or combinations of these measures.

Recent studies indicate that an increase in pro-inflammatory cytokines is seen in diverse cardiac diseases, including congestive heart failure, cardiomyopathy, and myocarditis. Hegewisch S, et al. *Lancet* 1990;2:294-295; Levine B, et al., *N.Engl.J.Med.* 1990;323 (4):236-241; Mann DL, et al., *Chest* 1994;105:897-904; and Givertz MM, et al., *Lancet* 1998;352:34-38 For example, the cytokine tumor necrosis factor- $\alpha$  (TNF) is synthesized by human cardiac myocytes, and the level of TNF expression correlates with the degree of cardiac dysfunction in patients. Torre-Amione G, et al., *J.Am.Coll.Cardiol.* 1996;27:1201-1206; Torre-Amione G, RD, et al. *Circulation* 1995;92:1487-1493; and Torre-Amione G, et al., *Circulation* 1996;93:704-711 In

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animals, synthesis of TNF by the heart is itself sufficient to cause cardiomyopathy and lethal cardiac failure. Bryant D, et al., *Circ.* 1998;97:175-183 and Kubota T, et al. *J.Am.Coll.Cardiol.* 1997;346A(Abstract) Furthermore, early human trials have demonstrated that antagonism of TNF improves cardiac failure in humans with NYHA  
5 Class III heart failure or idiopathic dilated cardiomyopathy. Deswal et al., *Circulation* 96: I-323 (1997); and Sliwa et al., *Lancet* 351: 1091-1093 (1998) However, the primary stimulus for cytokine secretion remains unknown.

Bacterial endotoxin, or lipopolysaccharide (LPS), is a primary inducer of TNF production during sepsis. With respect to cardiac diseases, the role of endotoxin has  
10 been examined primarily in the context of cardiopulmonary bypass, driven by the hypothesis that endotoxin may be present in the extracorporeal circuit, or may be translocated across the intestine secondary to non-pulsatile, low flow perfusion. Riddington DW, et al. *JAMA* 1996;275:1007-1012 and Wan S, et al., *Chest* 1997;112:676-692 These studies have demonstrated only transient low-level  
15 endotoxemia during cardiopulmonary bypass, with rapid resolution following completion of cardiopulmonary bypass in the majority of patients. Nilsson L, *J Thorac Cardiovasc Surg* 1990;100:777-780; Casey WF, *Crit.Care Med.* 1992;20 (8):1090-1096; Khabar KS, et al., *Clin Immunol Immunopathol* 1997;85:97-103; Jansen NJ, *Ann Thorac Surg* 1992;54:744-747. Bennett-Guerrero E et al., *JAMA* 1997;277:646-650 reported that  
20 lower levels of anti-endotoxin antibodies pre-operatively were associated with an increased risk of post-operative complications and hypothesized that this difference was due to a poor immunity to endotoxin.

Investigators have thus far failed to demonstrate, or failed to attempt to demonstrate, persistent endotoxemia in a majority of patients with cardiac disease  
25 Nilsson L, *J Thorac Cardiovasc Surg* 1990;100:777-780; Casey WF, *Crit.Care Med.* 1992;20 (8):1090-1096; Khabar KS, et al., *Clin Immunol Immunopathol* 1997;85:97-103; Jansen NJ, *Ann Thorac Surg* 1992;54:744-747. See also Niebauer J, *Eur. Heart J.* 1998;19:174, which reported elevated levels of plasma endotoxin in adults with edematous chronic congestive heart failure that was not associated with elevated levels  
30 of LBP or anti-endotoxin antibodies (indicators of long-term endotoxin exposure).

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BPI is a protein isolated from the granules of mammalian polymorphonuclear leukocytes (PMNs or neutrophils), which are blood cells essential in the defense against invading microorganisms. Human BPI protein has been isolated from PMNs by acid extraction combined with either ion exchange chromatography [Elsbach, *J. Biol. Chem.*, 254:11000 (1979)] or *E. coli* affinity chromatography [Weiss, et al., *Blood*, 69:652 (1987)]. BPI obtained in such a manner is referred to herein as natural BPI and has been shown to have potent bactericidal activity against a broad spectrum of gram-negative bacteria. The molecular weight of human BPI is approximately 55,000 daltons (55 kD). The amino acid sequence of the entire human BPI protein and the nucleic acid sequence of DNA encoding the protein have been reported in Figure 1 of Gray et al., *J. Biol. Chem.*, 264:9505 (1989), incorporated herein by reference. The Gray et al. amino acid sequence is set out in SEQ ID NO: 1 hereto. U.S. Patent No. 5,198,541 discloses recombinant genes encoding and methods for expression of BPI proteins, including BPI holoprotein and fragments of BPI.

BPI is a strongly cationic protein. The N-terminal half of BPI accounts for the high net positive charge; the C-terminal half of the molecule has a net charge of -3. [Elsbach and Weiss (1981), *supra*.] A proteolytic N-terminal fragment of BPI having a molecular weight of about 25 kD possesses essentially all the anti-bacterial efficacy of the naturally-derived 55 kD human BPI holoprotein. [Ooi et al., *J. Bio. Chem.*, 262: 14891-14894 (1987)]. In contrast to the N-terminal portion, the C-terminal region of the isolated human BPI protein displays only slightly detectable anti-bacterial activity against gram-negative organisms. [Ooi et al., *J. Exp. Med.*, 174:649 (1991).] An N-terminal BPI fragment of approximately 23 kD, referred to as "rBPI<sub>23</sub>," has been produced by recombinant means and also retains anti-bacterial activity against gram-negative organisms. [Gazzano-Santoro et al., *Infect. Immun.* 60:4754-4761 (1992).] An N-terminal analog of BPI, rBPI<sub>21</sub>, has been produced as described in Horwitz et al., *Protein Expression Purification*, 8:28-40 (1996).

The bactericidal effect of BPI was originally reported to be highly specific to gram-negative species, e.g., in Elsbach and Weiss, *Inflammation: Basic Principles and Clinical Correlates*, eds. Gallin et al., Chapter 30, Raven Press, Ltd. (1992). The precise mechanism by which BPI kills gram-negative bacteria is not yet completely



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elucidated, but it is believed that BPI must first bind to the surface of the bacteria through electrostatic and hydrophobic interactions between the cationic BPI protein and negatively charged sites on LPS. In susceptible gram-negative bacteria, BPI binding is thought to disrupt LPS structure, leading to activation of bacterial enzymes that degrade phospholipids and peptidoglycans, altering the permeability of the cell's outer membrane, and initiating events that ultimately lead to cell death. [Elsbach and Weiss (1992), *supra*]. LPS has been referred to as "endotoxin" because of the potent inflammatory response that it stimulates, i.e., the release of mediators by host inflammatory cells which may ultimately result in irreversible endotoxic shock. BPI binds to lipid A, reported to be the most toxic and most biologically active component of LPS.

BPI protein products have a wide variety of beneficial activities. BPI protein products are bactericidal for gram-negative bacteria, as described in U.S. Patent Nos. 5,198,541 and 5,523,288, both of which are incorporated herein by reference. International Publication No. WO 94/20130 (incorporated herein by reference) proposes methods for treating subjects suffering from an infection (*e.g.* gastrointestinal) with a species from the gram-negative bacterial genus *Helicobacter* with BPI protein products. BPI protein products also enhance the effectiveness of antibiotic therapy in gram-negative bacterial infections, as described in U.S. Patent No. 5,523,288 and International Publication No. WO 95/08344 (PCT/US94/11255), which are incorporated herein by reference. BPI protein products are also bactericidal for gram-positive bacteria and mycoplasma, and enhance the effectiveness of antibiotics in gram-positive bacterial infections, as described in U.S. Patent Nos. 5,578,572 and 5,783,561 and International Publication No. WO 95/19180 (PCT/US95/00656), which are incorporated herein by reference. BPI protein products exhibit anti-fungal activity, and enhance the activity of other anti-fungal agents, as described in U.S. Patent No. 5,627,153 and International Publication No. WO 95/19179 (PCT/US95/00498), and further as described for anti-fungal peptides in U.S. Patent No. 5,858,974, which is in turn a continuation-in-part of U.S. Application Serial No. 08/504,841 filed July 20, 1994 and corresponding International Publication Nos. WO 96/08509 (PCT/US95/09262) and WO 97/04008 (PCT/US96/03845), all of which are incorporated herein by reference. BPI protein products exhibit anti-protozoan activity, as described in U.S. Patent No. 5,646,114 and

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International Publication No. WO 96/01647 (PCT/US95/08624), which are incorporated herein by reference. BPI protein products exhibit anti-chlamydial activity, as described in co-owned, co-pending U.S. Application Serial No. 08/694,843 filed August 9, 1996 and WO 98/06415 (PCT/US97/13810), which are incorporated herein by reference.

5 Finally, BPI protein products exhibit anti-mycobacterial activity, as described in co-owned, co-pending U.S. Application Serial No. 08/626,646 filed April 1, 1996, which is in turn a continuation of U.S. Application Serial No. 08/285,803 filed August 14, 1994, which is in turn a continuation-in-part of U.S. Application Serial No. 08/031,145 filed March 12, 1993 and corresponding International Publication No. WO94/20129

10 (PCT/US94/02463), all of which are incorporated herein by reference.

The effects of BPI protein products in humans with endotoxin in circulation, including effects on TNF, IL-6 and endotoxin are described in U.S. Patent No. 5,643,875, which is incorporated herein by reference.

BPI protein products are also useful for treatment of specific disease

15 conditions, such as meningococcemia in humans (as described in co-owned, co-pending U.S. Application Serial No. 08/644,287 filed May 10, 1996 and continuation No. 08/927,437 filed September 10, 1997 and International Publication No. WO97/42966 (PCT/US97/08016), all of which are incorporated herein by reference), hemorrhagic trauma in humans, (as described in U.S. Patent No. 5,756,464, U.S. Application Serial

20 No. 08/862,785 filed May 23, 1997 and corresponding International Publication No. WO 97/44056 (PCT/US97/08941), all of which are incorporated herein by reference), burn injury (as described in U.S. Patent No. 5,494,896, which is incorporated herein by reference), ischemia/reperfusion injury (as described in U.S. Patent No. 5,578,568, incorporated herein by reference), and liver resection (as described in co-owned, co-

25 pending U.S. Application Serial No. 08/582,230 filed January 3, 1996, which is in turn a continuation of U.S. Application Serial No. 08/318,357 filed October 5, 1994, which is in turn a continuation-in-part of U.S. Application Serial No. 08/132,510 filed October 5, 1993, and corresponding International Publication No. WO 95/10297 (PCT/US94/11404), all of which are incorporated herein by reference).

30 BPI protein products also neutralize the anti-coagulant activity of exogenous heparin, as described in U.S. Patent No. 5,348,942, incorporated herein by

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reference, and are useful for treating chronic inflammatory diseases such as rheumatoid and reactive arthritis and for inhibiting angiogenesis and for treating angiogenesis-associated disorders including malignant tumors, ocular retinopathy and endometriosis, as described in U.S. Patent Nos. 5,639,727, 5,807,818 and 5,837,678 and International  
5 Publication No. WO 94/20128 (PCT/US94/02401), all of which are incorporated herein by reference.

BPI protein products are also useful in antithrombotic methods, as described in U.S. Patent No. 5,741,779 and U.S. Application Serial No. 09/063,465 filed April 20, 1998 and corresponding WO 97/42967 (PCT/US7/08017), all of which are  
10 incorporated herein by reference.

### SUMMARY OF THE INVENTION

The present invention provides novel therapeutic uses for BPI protein products that involve treatment of subjects with chronic cardiac disease. Uses of BPI  
15 protein products according to the invention are specifically contemplated for prophylactic or therapeutic treatment of chronic cardiac disease states or conditions in humans, particularly humans with chronic cardiac disease who exhibit elevated levels of circulating LPS and circulating LBP (in plasma or serum). Chronic cardiac disease states or conditions include but are not limited to cardiomyopathies, chronic congestive heart  
20 failure, and congenital heart defects.

Chronic congestive heart failure as used herein includes long-term congestive heart failure (*i.e.*, congestive heart failure persisting more than two weeks, or more than three weeks, or more than one month, or more than two months, or more than three months), congestive heart failure that persists after correction of reversible causes,  
25 and congestive heart failure not immediately associated with an acute myocardial infarction or an acute infectious process.

Congenital heart defects, which may result in congestive heart failure or cyanotic heart disease, include pulmonary atresia, total anomalous pulmonary venous return, ventricular septal defect, hypoplastic left heart syndrome, double outlet right  
30 ventricle, right pulmonary artery stenosis, interrupted aortic arch, Ebsteins's anomaly,

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tetralogy of Fallot, atrioventricular canal, transposition of the great arteries and truncus arteriosus.

It is contemplated that the administration of a BPI protein product may be accompanied by the concurrent administration of other known therapeutic agents for treating the chronic cardiac disease state. For example, agents that are known in the art for treating congestive heart failure include diuretics (including thiazides such as chlorothiazide, hydrochlorothiazide and metolazone, loop diuretics such as ethacrynic acid, furosemide, torsemide and bumetanide and their congeners, potassium sparing agents such as spironolactone, canrenone, triamterene and amiloride, and others such as metolazone and other quinazoline-sulfonamides), vasodilators (including nitrovasodilators such as nitroglycerin, isosorbide dinitrate, and sodium nitroprusside, hydralazine, prostacyclin, ACE inhibitors such as captopril, enalapril, lisinopril, quinapril and ramipril, and angiotensin II antagonists such as losartan), positive inotropic agents (such as cardiac glycosides, including digitalis or digoxin), phosphodiesterase inhibitors (such as amrinone and milrinone, primarily useful for short term support), occasionally beta-adrenergic receptor antagonists (beta blockers such as propranolol, metoprolol, atenolol, pindolol, acebutolol, labetalol, carvedilol and celiprolol), or combinations of these measures. See, e.g., Goodman and Gilman, Ch. 34, The Pharmacological Basis of Therapeutics, McGraw Hill, New York (1996), incorporated by reference herein.

The invention also contemplates use of a BPI protein product in the preparation of a medicament for the prophylactic or therapeutic treatment of a chronic cardiac disease state.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently preferred embodiments thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A displays plasma LPS levels for all patients completing the study protocol (n=29). Figure 1B displays data from patients with endotoxemia pre-operatively (n=11), while Figure 1C displays data from patients without endotoxemia pre-operatively (n=18).

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Figure 2 displays plasma LBP levels from all patients completing the study protocol (n=29).

Figure 3 displays plasma IL-6 levels from all patients completing the study protocol (n=29).

5                   Figures 4A and 4B displays pre-operative plasma LPS (Fig. 4B) and LBP (Fig. 4A) levels in patients with a severe (n=15), versus less severe (n=15), post-operative clinical course.

### DETAILED DESCRIPTION OF THE INVENTION

10                   The present invention provides novel therapeutic uses for BPI protein products, particularly BPI-derived peptides, that involve treatment of chronic cardiac disease. "Treatment" as used herein encompasses both prophylactic and therapeutic treatment. The invention contemplates methods for treatment of subjects suffering from chronic heart disease which comprise the administration of therapeutically effective  
15                   amounts of bactericidal/permeability-increasing protein (BPI) protein products to those subjects so as to alleviate the negative physiological effects of endotoxemia.

                  The invention is based on the discovery that a substantial proportion of subjects suffering from chronic heart disease exhibit evidence of endotoxemia associated with the chronic heart disease prior to surgery, and that this endotoxemia correlates to a  
20                   poorer prognosis for these subjects. Thus, one basis for the invention is the expectation that endotoxemia is not simply a side effect of chronic cardiac disease but is a significant contributing factor to the pathology of chronic cardiac disease.

                  Therapeutic uses of BPI protein products are specifically contemplated for treatment of mammals, including humans, suffering from chronic cardiac disease as  
25                   distinguished from acute cardiac disease states such as myocardial infarction, circulatory collapse from hemorrhage and the like.

                  Another aspect of the present invention is the treatment of patients undergoing cardiopulmonary bypass, on the basis that the severity of endotoxemia during and after cardiopulmonary bypass is correlated with poorer post-surgical outcome. Thus,  
30                   treatment with BPI protein product is expected to improve post-surgical outcome.

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As used herein, "BPI protein product" includes naturally and recombinantly produced BPI protein; natural, synthetic, and recombinant biologically active polypeptide fragments of BPI protein; biologically active polypeptide variants of BPI protein or fragments thereof, including hybrid fusion proteins and dimers; 5 biologically active polypeptide analogs of BPI protein or fragments or variants thereof, including cysteine-substituted analogs; and BPI-derived peptides. The BPI protein products administered according to this invention may be generated and/or isolated by any means known in the art. U.S. Patent No. 5,198,541, the disclosure of which is incorporated herein by reference, discloses recombinant genes encoding, and methods for 10 expression of, BPI proteins including recombinant BPI holoprotein, referred to as rBPI and recombinant fragments of BPI. U.S. Patent No. 5,439,807 and corresponding International Publication No. WO 93/23540 (PCT/US93/04752), which are all incorporated herein by reference, disclose novel methods for the purification of recombinant BPI protein products expressed in and secreted from genetically transformed 15 mammalian host cells in culture and discloses how one may produce large quantities of recombinant BPI products suitable for incorporation into stable, homogeneous pharmaceutical preparations.

Biologically active fragments of BPI (BPI fragments) include biologically active molecules that have the same or similar amino acid sequence as a natural human 20 BPI holoprotein, except that the fragment molecule lacks amino-terminal amino acids, internal amino acids, and/or carboxy-terminal amino acids of the holoprotein. Nonlimiting examples of such fragments include an N-terminal fragment of natural human BPI of approximately 25 kD, described in Ooi et al., *J. Exp. Med.*, 174:649 (1991), and the recombinant expression product of DNA encoding N-terminal amino 25 acids from 1 to about 193 to 199 of natural human BPI, described in Gazzano-Santoro et al., *Infect. Immun.* 60:4754-4761 (1992), and referred to as rBPI<sub>23</sub>. In that publication, an expression vector was used as a source of DNA encoding a recombinant expression product (rBPI<sub>23</sub>) having the 31-residue signal sequence and the first 199 amino acids of the N-terminus of the mature human BPI, as set out in Figure 1 of Gray et al., *supra*, 30 except that valine at position 151 is specified by GTG rather than GTC and residue 185 is glutamic acid (specified by GAG) rather than lysine (specified by AAG). Recombinant

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holoprotein (rBPI) has also been produced having the sequence (SEQ ID NOS: 1 and 2) set out in Figure 1 of Gray et al., *supra*, with the exceptions noted for rBPI<sub>23</sub> and with the exception that residue 417 is alanine (specified by GCT) rather than valine (specified by GTT). A fragment consisting of residues 10-193 of BPI has been described in co-owned, co-pending U.S. Application Serial No. 09/099,725 filed June 19, 1998, incorporated herein by reference. Other examples include dimeric forms of BPI fragments, as described in U.S. Patent No. 5,447,913 and corresponding International Publication No. WO 95/24209 (PCT/US95/03125), all of which are incorporated herein by reference.

Biologically active variants of BPI (BPI variants) include but are not limited to recombinant hybrid fusion proteins, comprising BPI holoprotein or biologically active fragment thereof and at least a portion of at least one other polypeptide, and dimeric forms of BPI variants. Examples of such hybrid fusion proteins and dimeric forms are described in U.S. Patent No. 5,643,570 and corresponding International Publication No. WO 93/23434 (PCT/US93/04754), which are all incorporated herein by reference and include hybrid fusion proteins comprising, at the amino-terminal end, a BPI protein or a biologically active fragment thereof and, at the carboxy-terminal end, at least one constant domain of an immunoglobulin heavy chain or allelic variant thereof.

Biologically active analogs of BPI (BPI analogs) include but are not limited to BPI protein products wherein one or more amino acid residues have been replaced by a different amino acid. For example, U.S. Patent No. 5,420,019 and corresponding International Publication No. WO 94/18323 (PCT/US94/01235), all of which are incorporated herein by reference, discloses polypeptide analogs of BPI and BPI fragments wherein a cysteine residue is replaced by a different amino acid. A stable BPI protein product described by this application is the expression product of DNA encoding from amino acid 1 to approximately 193 or 199 of the N-terminal amino acids of BPI holoprotein, but wherein the cysteine at residue number 132 is substituted with alanine and is designated rBPI<sub>21</sub>Δcys or rBPI<sub>21</sub>. Production of this N-terminal analog of BPI, rBPI<sub>21</sub>, has been described in Horwitz et al., *Protein Expression Purification*, 8:28-40 (1996). Similarly, a fragment consisting of residues 10-193 of BPI in which the cysteine at position 132 is replaced with an alanine (designated "rBPI(10-193)C132A" or "rBPI(10-193)ala<sup>132</sup>") has been described in co-owned, co-pending U.S. Application

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Serial No. 09/099,725 filed June 19, 1998. Other examples include dimeric forms of BPI analogs; e.g. U.S. Patent No. 5,447,913 and corresponding International Publication No. WO 95/24209 (PCT/US95/03125), all of which are incorporated herein by reference.

Other BPI protein products useful according to the methods of the invention are peptides derived from or based on BPI produced by synthetic or recombinant means (BPI-derived peptides), such as those described in International Publication No. WO 97/04008 (PCT/US96/03845), which corresponds to U.S. Application Serial No. 08/621,259 filed March 21, 1996, and International Publication No. WO 96/08509 (PCT/US95/09262), which corresponds to U.S. Patent No. 5,858,974, and International Publication No. WO 95/19372 (PCT/US94/10427), which corresponds to U.S. Patent No. 5,652,332, and International Publication No. WO94/20532 (PCT/US94/02465), which corresponds to U.S. Patent No. 5,763,567 which is a continuation of U.S. Patent No. 5,733,872, which is a continuation-in-part of U.S. Application Serial No. 08/183,222, filed January 14, 1994, which is a continuation-in-part of U.S. Application Serial No. 08/093,202 filed July 15, 1993 (corresponding to International Publication No. WO 94/20128 (PCT/US94/02401)), which is a continuation-in-part of U.S. Patent No. 5,348,942, as well as International Application No. PCT/US97/05287, which corresponds to U.S. Patent No. 5,851,802, the disclosures of all of which are incorporated herein by reference.

Presently preferred BPI protein products include recombinantly-produced N-terminal analogs and fragments of BPI, especially those having a molecular weight of approximately between 20 to 25 kD such as rBPI<sub>21</sub> or rBPI<sub>23</sub>, rBPI(10-193)C132A (rBPI(10-193)ala<sup>132</sup>), dimeric forms of these N-terminal proteins (e.g., rBPI<sub>42</sub> dimer), and BPI-derived peptides.

The administration of BPI protein products is preferably accomplished with a pharmaceutical composition comprising a BPI protein product and a pharmaceutically acceptable diluent, adjuvant, or carrier. The BPI protein product may be administered without or in conjunction with known surfactants or other therapeutic agents. A stable pharmaceutical composition containing BPI protein products (e.g., rBPI<sub>23</sub>) comprises the BPI protein product at a concentration of 1 mg/ml in citrate buffered saline (5 or 20 mM citrate, 150 mM NaCl, pH 5.0) comprising 0.1% by weight



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of poloxamer 188 (Pluronic F-68, BASF Wyandotte, Parsippany, NJ) and 0.002% by weight of polysorbate 80 (Tween 80, ICI Americas Inc., Wilmington, DE). Another stable pharmaceutical composition containing BPI protein products (e.g., rBPI<sub>21</sub>) comprises the BPI protein product at a concentration of 2 mg/ml in 5 mM citrate, 150 mM NaCl, 0.2% poloxamer 188 and 0.002% polysorbate 80. Such preferred combinations are described in U.S. Patent Nos. 5,488,034 and 5,696,090 and corresponding International Publication No. WO 94/17819 (PCT/US94/01239), the disclosures of all of which are incorporated herein by reference. As described in U.S. Application Serial No. 08/586,133 filed January 12, 1996, which is in turn a continuation-in-part of U.S. Application Serial No. 08/530,599 filed September 19, 1995, which is in turn a continuation-in-part of U.S. Application Serial No. 08/372,104 filed January 13, 1995, and corresponding International Publication No. WO96/21436 (PCT/US96/01095), all of which are incorporated herein by reference, other poloxamer formulations of BPI protein products with enhanced activity may be utilized.

Therapeutic compositions comprising BPI protein product may be administered systemically or topically. Systemic routes of administration include oral, intravenous, intramuscular or subcutaneous injection (including into a depot for long-term release), intraocular and retrobulbar, intrathecal, intraperitoneal (e.g. by intraperitoneal lavage), intrapulmonary (using powdered drug, or an aerosolized or nebulized drug solution), or transdermal.

When given parenterally, BPI protein product compositions are generally injected in doses ranging from 1 µg/kg to 100 mg/kg per day, preferably at doses ranging from 0.1 mg/kg to 20 mg/kg per day, more preferably at doses ranging from 1 to 20 mg/kg/day and most preferably at doses ranging from 2 to 10 mg/kg/day. The treatment may continue by continuous infusion or intermittent injection or infusion, at the same, reduced or increased dose per day for, e.g., 1 to 3 days, and additionally as determined by the treating physician. When administered intravenously, BPI protein products are preferably administered by an initial brief infusion followed by a continuous infusion. The preferred intravenous regimen is a 1 to 20 mg/kg brief intravenous infusion of BPI protein product followed by a continuous intravenous infusion at a dose of 1 to 20 mg/kg/day, continuing for up to one week. A particularly preferred intravenous dosing

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regimen is a 1 to 4 mg/kg initial brief intravenous infusion followed by a continuous intravenous infusion at a dose of 1 to 4 mg/kg/day, continuing for up to 72 hours.

Those skilled in the art can readily optimize effective dosages and administration regimens for therapeutic compositions comprising BPI protein product, as determined by good medical practice and the clinical condition of the individual patient.

"Concurrent administration," or "co-administration," as used herein includes administration of the agents, in conjunction or combination, together, or before or after each other. The BPI protein product and second agent(s) may be administered by different routes. For example, the BPI protein product may be administered intravenously while the second agent(s) is(are) administered intravenously, intramuscularly, subcutaneously, orally or intraperitoneally. The BPI protein product and second agent(s) may be given sequentially in the same intravenous line or may be given in different intravenous lines. Alternatively, the BPI protein product may be administered in a special form for gastric delivery, while the second agent(s) is(are) administered, e.g., orally. The formulated BPI protein product and second agent(s) may be administered simultaneously or sequentially, as long as they are given in a manner sufficient to allow all agents to achieve effective concentrations at the site of action.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 addresses a study in which thirty children with complex chronic heart disease were tested for markers of endotoxemia prior to and at 1, 8, 24, 48 and 72 hours following cardiopulmonary bypass surgery.

#### EXAMPLE 1

The experimental protocol, approved by the Institutional Review Board at the University of Texas Southwestern Medical Center, was an unblinded, prospective study in which 30 children with severe congenital heart disease were sequentially enrolled while awaiting surgical repair and/or palliation. One patient with hypoplastic left heart syndrome died intra-operatively, and therefore data on this child are included

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only in the pre-operative analysis. Patients with clinical evidence of preoperative infection were excluded from the study.

The 30 enrolled children ranged in age from 4 days to 402 days (median age 59 days), and in weight from 2.0 to 9.5 kg (median weight 4.0 kg) The genders, ages, cardiac diagnoses, and surgical repairs are listed in Table 1 below.

5

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Table 1. Patient Characteristics

	<u>Sex</u>	<u>Age-days</u>	<u>Wt (kg)</u>	<u>Diagnosis*</u>	<u>Procedure</u>
	M	349	8.90	Pulmonary Atresia	RVOT Reconstruction
	M	6	5.70	TAPVR	TAPVR Repair
5	F	265	6.60	TAPVR	TAPVR Repair
	M	5	3.30	Interrupted Aortic Arch	Aortic Arch Repair
	M	44	3.50	Ebstein's anomaly, VSD	VSD Repair
	M	245	8.09	VSD	VSD Repair
	F	7	2.50	HLHS	Norwood Procedure
10	F	10	3.4	HLHS	Norwood Procedure
	F	210	6.70	VSD	VSD Repair
	M	163	5.00	DORV, RPA Stenosis	DKS
	F	395	8.20	TOF	Tetralogy Repair
	M	10	2.50	TGA ; DORV	Arterial Switch
15	M	75	3.60	AV Canal	AV Canal Repair
	M	4	4.40	TGA	Arterial Switch
	M	4	3.60	TGA	Arterial Switch
	M	4	3.35	LV Rhabdomyosarcoma	Tumor Resection
	M	26	3.70	TAPVR	TAPVR Repair
20	F	110	3.20	TAPVR	TAPVR Repair
	F	126	2.00	Truncus Arteriosus	RVOT Reconstruction
	M	402	9.50	TOF	Tetralogy Repair
	M	25	2.60	Pulmonary Atresia	RVOT Reconstruction
	F	105	4.40	AV Canal	AV Canal Repair
25	M	178	5.80	TOF	Tetralogy Repair
	M	5	4.40	TGA	Arterial Switch
	M	102	4.10	TAPVR	TAPVR Repair
	F	5	3.70	TAPVR	TAPVR Repair
	M	6	3.10	HLHS	Norwood Procedure
30	F	120	5.40	AV Canal	AV Canal Repair
	M	4	3.80	TGA	Arterial Switch
	M	335	7.50	TOF	Tetralogy Repair

35 \*RVOT-right ventricular outflow tract; TAPVR-total anomalous pulmonary venous return; VSD-ventricular septal defect; HLHS-hypoplastic left heart syndrome; DORV-double outlet right ventricle; RPA-right pulmonary artery; DKS-Damus-Kaye-Stansel shunt operation; TOF-tetralogy of Fallot; AV canal-atrioventricular canal; TGA-transposition of the great arteries.

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Anesthesia was induced with sevoflurane, nitrous oxide and oxygen; intubation was facilitated with intravenous rocuronium and fentanyl. Anesthesia was maintained with fentanyl (30-50 mcg/kg), isoflurane, and pancuronium. Nine patients received tranexamic acid (50-100 mg/kg) and 3 received aprotinin (dosed to achieve 350 units/mL total blood volume).

Cardiopulmonary bypass was performed as follows. The extracorporeal circuit consisted of a roller pump, membrane oxygenator, and cardiotomy filters. Prior to the institution of cardiopulmonary bypass, the patients' blood was anticoagulated with heparin (300 units/kg). 13 patients underwent deep hypothermic circulatory arrest (core temp 16-18°C) and the remainder were cooled to a core temperature of 25-30°C for the completion of surgery. Hemofiltration was performed prior to completion of cardiopulmonary bypass on all patients in an attempt to remove excess free water and attain a hemoglobin >12 gm/dL.

Blood samples for the determination of LPS, LBP, and IL-6 were obtained prior to surgery and at 1, 8, 24, 48 and 72 hours after completion of cardiopulmonary bypass. The pre-operative sample was obtained from a newly placed central venous catheter, immediately after the induction of anesthesia and endotracheal intubation. For determination of endotoxin levels, blood samples were collected into heparinized Vacutainer™ tubes (Becton-Dickson, Rutherford NJ) selected for low endotoxin content (BioWhittaker, Walkersville, MD), immediately placed on ice, and walked to the laboratory by an investigator. Platelet-rich plasma was obtained by centrifugation (180 x g, 10 min, 2-8°C). Samples were stored at -70°C until assay.

LPS, LBP and IL-6 assays were conducted in a blinded fashion. The level of LPS in the platelet-rich plasma was determined by using a kinetic chromogenic Limulus amoebocyte lysate assay (Endochrome-K™, Endosafe, Charleston, SC) according to the manufacturer's instructions. LPS concentrations are expressed in terms of endotoxin units (EU) per ml relative to an *E. coli* O55:B5 control standard endotoxin. LBP levels were determined by ELISA as described in Meszaros S, et al., *Infect. Immun.* 1995;63:363-365. IL-6 was measured using a sandwich ELISA (R&D Systems, Minneapolis).

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A severe (*versus* less-severe) post operative course was prospectively defined. Severity of post-operative myocardial dysfunction was estimated according to an adaptation of the scale utilized by Wernovsky et al., *Circulation* 1995;92:2226-2235. Specifically, an inotropic support score was calculated as follows: each 1.0 mcg/kg/min of dopamine or dobutamine, and each 0.01 mcg/kg/min of epinephrine yielded a score of 1. Children with a net positive fluid balance of >40 cc/kg in the first 24 hours *and* an inotropic support score of >12, *or* perioperative death, were considered to have a severe clinical course. Post-operative severity of illness was scored prior to knowledge of LPS, LBP, or IL-6 values.

10 All statistical analyses were performed on the Statistical Package for the Social Sciences (SPSS). Wilcoxon signed rank tests for non-parametric data were performed to determine if a significant rise in LPS, LBP, or IL-6 had occurred post-operatively. A Mann-Whitney test for non-parametric data was performed to determine if there was a significant difference in LPS or LBP concentrations between the patients  
15 who had a more severe clinical course compared to those with a less severe clinical course.

Twenty-nine of the thirty patients (96%) had evidence of endotoxemia during the study period, either by detection of elevated LPS directly or by detection of an elevated LBP plasma level > 2SD above the mean for healthy adults. The LPS, LBP and  
20 IL-6 levels for all patients are displayed in Figures 1A, 2 and 3. Figure 1A displays data from all children completing the study protocol and demonstrate elevated LPS at all time points. Differences between pre-operative and post-operative LPS levels are statistically non-significant. To better elucidate endotoxin kinetics, we divided patients into two groups: those who were endotoxemic prior to cardiopulmonary bypass, and those who  
25 were not endotoxemic prior to cardiopulmonary bypass (CPB). Levels for these two groups are shown in Figures 1B and 1C, respectively.

Prior to CPB, 12 patients had significant elevation of plasma endotoxin. In these patients, endotoxin tended to decline following completion of cardiopulmonary bypass, likely due to hemodilution / partial exchange transfusion (Figure 1B); but  
30 endotoxin levels remained abnormally elevated throughout the study period. In those children *without* pre-operative endotoxemia, the level of plasma endotoxin rose

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significantly following bypass, achieving a peak value at 1 hour post bypass, and remaining significantly elevated thereafter ( $p < 0.0001$ ) (Figure 1C).

There was a transient, but significant decrease in plasma LBP immediately after completion of cardiopulmonary bypass and hemofiltration. The decrease in plasma LBP at 1 hour following cardiopulmonary bypass was statistically significant ( $p < 0.0001$ ), and the increase in LBP at all points thereafter was also highly consistent and statistically significant ( $p < 0.0001$ ) compared to preoperative levels. This rise in LBP was similar for patients who were and were not endotoxemic preoperatively. Similarly, there was a significant rise in IL-6 at all time points following CPB ( $p < 0.05$ ) compared to preoperative levels (Figure 3).

Finally, it was determined whether children who experienced a more severe clinical course, defined prospectively, might differ from less severe patients when pre-operative LPS and LBP levels were compared. In this comparison, the more severely ill children had significantly higher pre-operative plasma LBP ( $p < 0.02$ ) (Figure 4A), and tended toward higher pre-operative LPS ( $p < 0.05$ ) compared to patients who experienced a less severe post-operative course (Figure 4B). Additionally, of the 12 patients who were endotoxemic prior to surgery, there were 3 deaths (25%), compared to 0 deaths in the 18 patients who were not endotoxemic prior to surgery ( $p = 0.054$ ).

The underlying biology of peri-operative endotoxemia is clarified by dividing those patients who were or were not endotoxemic pre-operatively. In patients who were endotoxemic pre-operatively, endotoxin levels initially fell following cardiopulmonary bypass, but remained abnormally elevated throughout the study period. This initial decrease may have been secondary to a dilution effect of cardiopulmonary bypass, given the infants' small blood volumes, or perhaps due to clearance of endotoxin by hemofiltration prior to completion of cardiopulmonary bypass. Millar AB, *Ann Thorac Surg* 1993;56:1499-1502 It is also possible that these patients, who were endotoxemic pre-operatively, may have induced and enhanced mechanisms for endotoxin clearance, compared to patients who were not endotoxemic pre-operatively. Dentener MA, et al., *Journal of Infectious Diseases* 1997;175:108-117  
Dentener MA, *Journal of Infectious Diseases* 1995;171:739-743; and Gazzano-Santoro et al., *Infect. Immunol.* 1994;62, No4:1185-1191

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In contrast, patients who were not endotoxemic pre-operatively demonstrated a significant elevation of plasma endotoxin at one and eight hours after cardiopulmonary bypass. A number of factors have been invoked to explain endotoxemia during cardiopulmonary bypass. First, there are many sources of endotoxin including the extracorporeal circuit, infusion solutions, drugs, and surgical materials. More importantly, increased intestinal permeability during cardiopulmonary bypass has been documented in adult patients, allowing for bacterial translocation and release of endotoxin into the circulation. Measures such as pulsatile perfusion or higher flow during bypass to improve gut perfusion and aggressive antibiotic regimens to decrease intestinal bacterial load prior to bypass have resulted in lower plasma LPS level. Watarida S, *et al.*, *J Thorac Cardiovasc Surg* 1994;108:620-625; and Quigley RL, *et al.* *Perfusion* 1995;10:27-31.

The finding of elevated LPS and LBP levels pre-operatively in a substantial proportion of children with chronic cardiac disease indicates that endotoxemia is associated with the chronic cardiac disease itself. The finding that severity of outcome correlated with endotoxemia as measured by elevated LPS and LBP levels indicates that measurement of endotoxemia can be used to predict prognosis. Consequently, the treatment of endotoxemia with BPI protein product is expected to ameliorate the signs and symptoms of chronic cardiac disease and to improve the prognosis of these patients.

Numerous modifications and variations of the above-described invention are expected to occur to those of skill in the art. Accordingly, only such limitations as appear in the appended claims should be placed thereon.



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## WHAT IS CLAIMED IS:

1. A method of treating a human with chronic cardiac disease comprising the step of administering a therapeutically effective amount of a bactericidal/permeability-increasing (BPI) protein product to said human.  
5
2. The method of claim 1 wherein the BPI protein product is rBPI<sub>21</sub>.
3. The method of claim 1 wherein the chronic cardiac disease is chronic congestive heart failure.  
10
4. The method of claim 1 wherein the chronic cardiac disease is cardiomyopathy.
5. The method of claim 1 wherein the chronic cardiac disease is a congenital heart defect.  
15
6. The method of claim 1 wherein the human exhibits elevated levels of circulating LPS.
7. The method of claim 1 wherein the human exhibits elevated levels of circulating LBP.  
20
8. The method of claim 1 wherein the human exhibits elevated levels of circulating LPS and circulating LBP.  
25
9. The method of claim 1 further comprising concurrently administering a second therapeutic agent for treating the chronic cardiac disease state.
10. The method of claim 9 wherein said chronic cardiac disease state is chronic congestive heart failure and the second therapeutic agent is selected from the group consisting of diuretics, positive inotropic agents, vasodilators and beta-blockers.  
30

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11. Use of a BPI protein product for the manufacture of a medicament for the treatment of a chronic cardiac disease state.

12. The use of claim 11 wherein the chronic cardiac disease is chronic  
5 congestive heart failure.

13. The use of claim 11 wherein the chronic cardiac disease is cardiomyopathy.

14. The use of claim 11 wherein the chronic cardiac disease is a  
10 congenital heart defect.

15. The use of claim 11 wherein the human exhibits elevated levels of circulating LPS and circulating LBP.  
15

16. Use of a BPI protein product for preparation of a medicament for treating a human suffering from a chronic cardiac disease state, wherein the human exhibits elevated levels of circulating LPS.

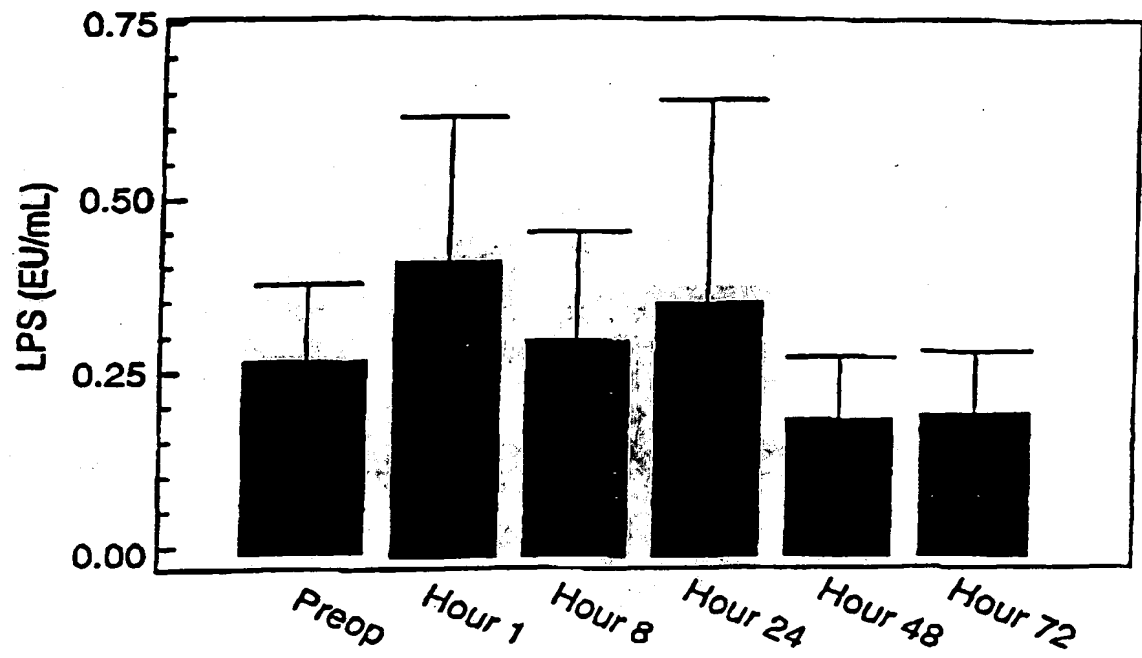
17. Use of a BPI protein product for preparation of a medicament for treating a human suffering from a chronic cardiac disease state, wherein the human exhibits elevated levels of circulating LBP.  
20

18. Use of a BPI protein product for preparation of a medicament for treating a human suffering from a chronic cardiac disease state, wherein the human exhibits elevated levels of circulating LPS and circulating LBP.  
25

19. Use of a BPI protein product for the preparation of a medicament for concurrent administration with another therapeutic agent for treating a chronic cardiac  
30 disease state.

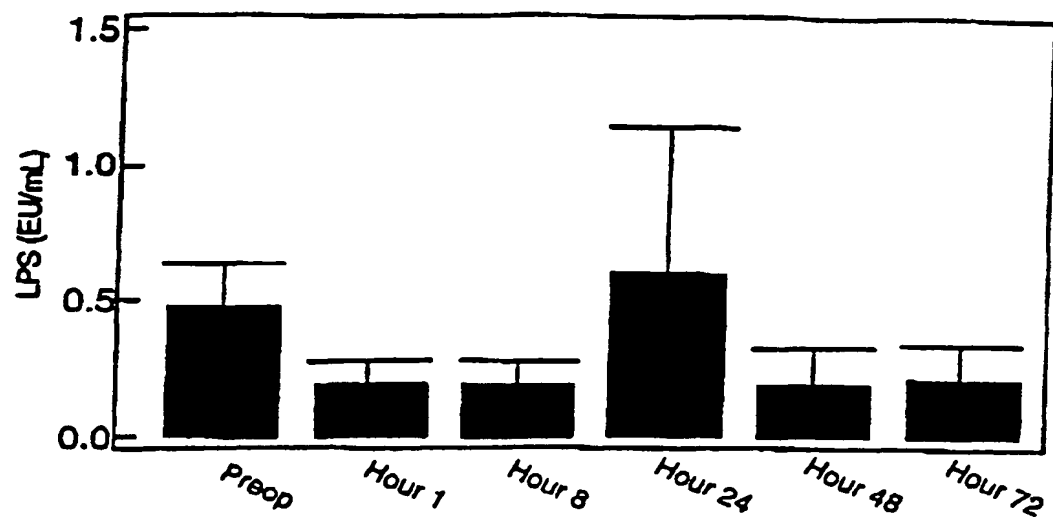
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Figure 1A



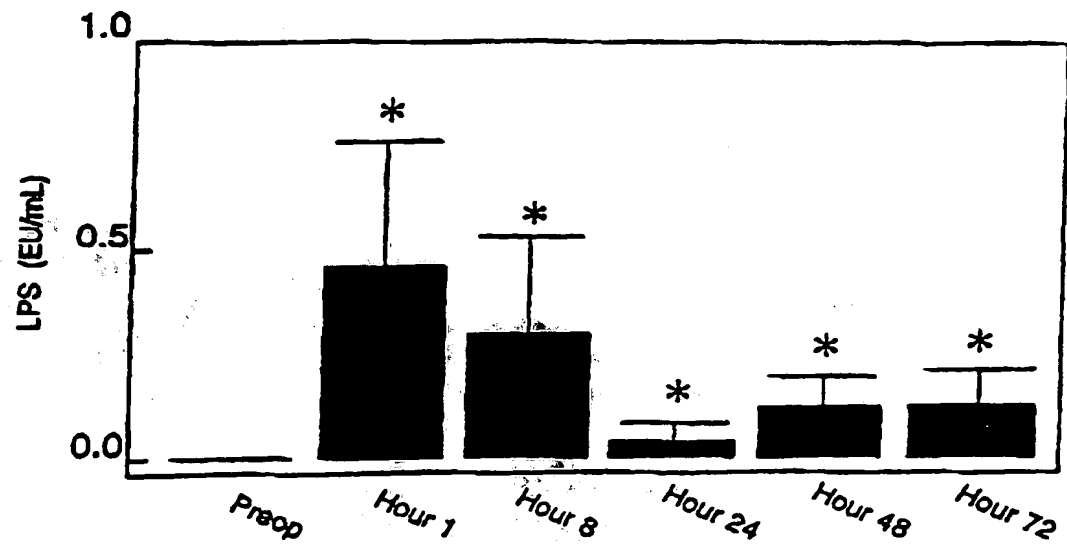
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Figure 1B



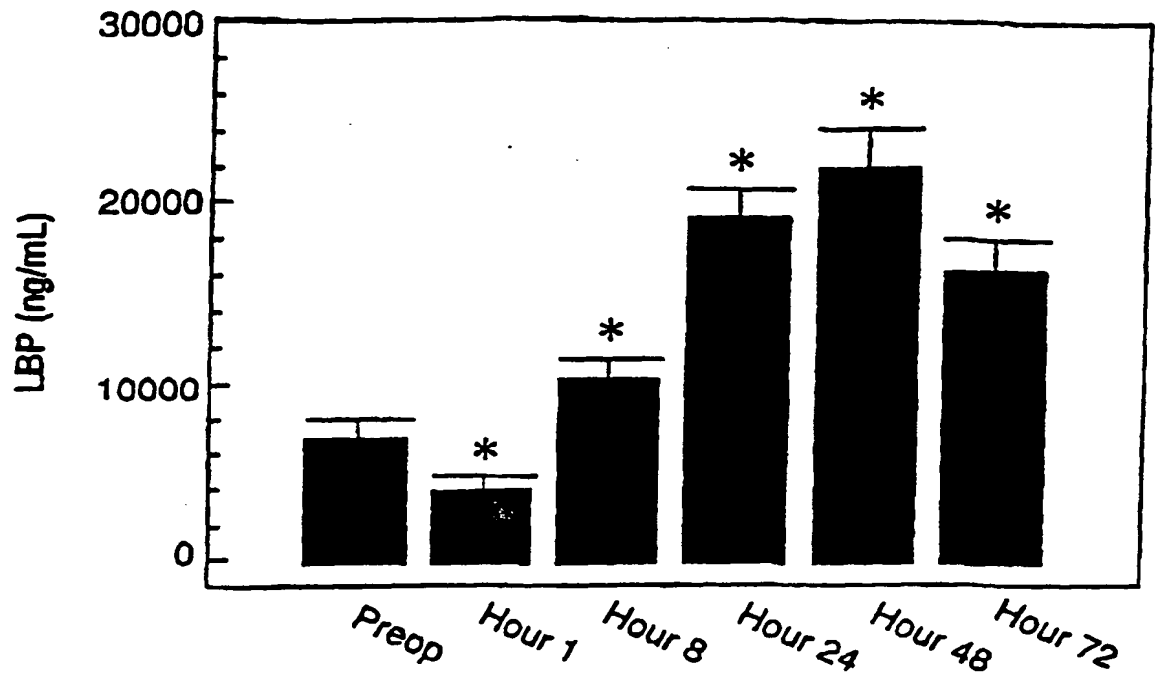
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Figure 1C



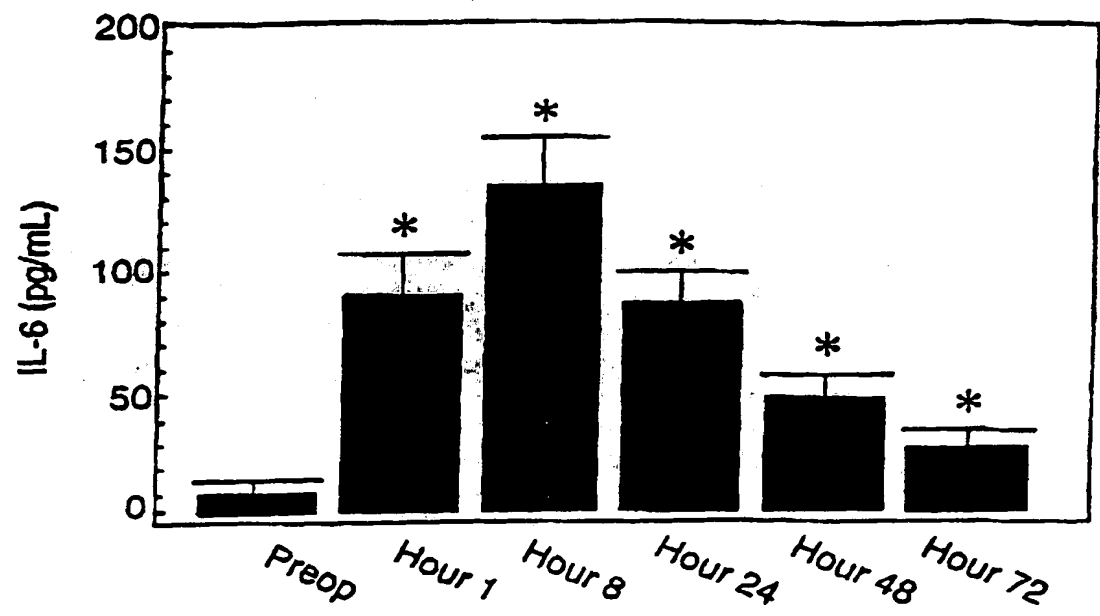
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Figure 2



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Figure 3



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Figure 4A

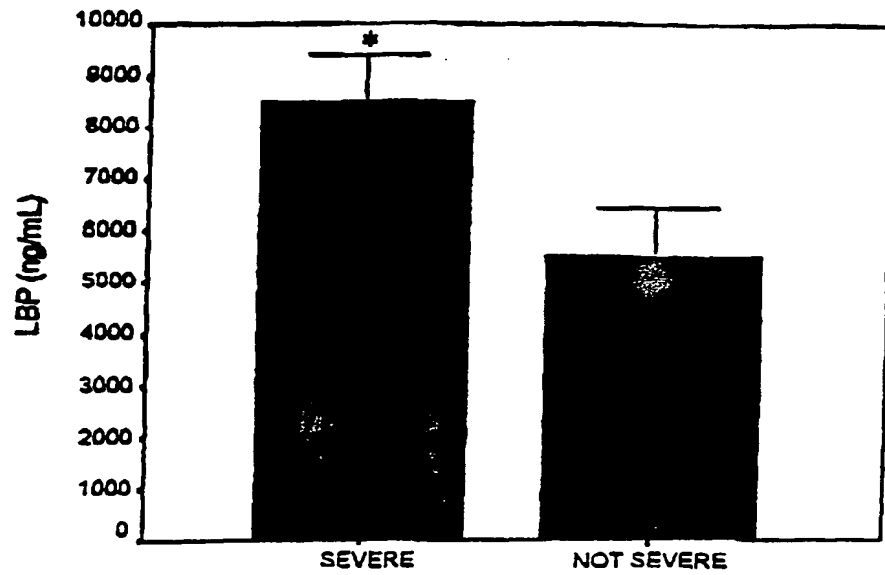
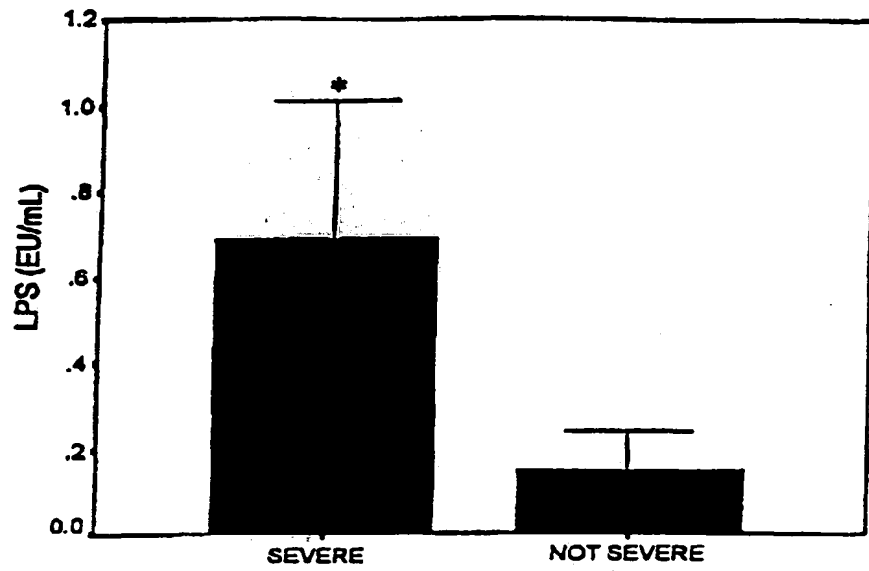




Figure 4B



- 1 -

## SEQUENCE LISTING

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BOARD OF REGENTS, UNIVERSITY OF TEXAS SYSTEM

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cctccaggaa tcgtgtttca attgtaacca agaaatttcc atttggtgctt catgaaaaaa 1791  
aacttctggt ttttttcatg tg 1813

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-30						-25					-20					
Ser	Leu	Met	Val	Leu	Val	Ala	Ile	Gly	Thr	Ala	Val	Thr	Ala	Ala	Val	
-15					-10					-5				-1	1	
Asn	Pro	Gly	Val	Val	Val	Arg	Ile	Ser	Gln	Lys	Gly	Leu	Asp	Tyr	Ala	
			5					10					15			
Ser	Gln	Gln	Gly	Thr	Ala	Ala	Leu	Gln	Lys	Glu	Leu	Lys	Arg	Ile	Lys	
		20					25					30				
Ile	Pro	Asp	Tyr	Ser	Asp	Ser	Phe	Lys	Ile	Lys	His	Leu	Gly	Lys	Gly	
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His	Tyr	Ser	Phe	Tyr	Ser	Met	Asp	Ile	Arg	Glu	Phe	Gln	Leu	Pro	Ser	
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		100					105					110				
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Ile	Thr	Cys	Ser	Ser	Cys	Ser	Ser	His	Ile	Asn	Ser	Val	His	Val	His	
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Ile	Ser	Lys	Ser	Lys	Val	Gly	Trp	Leu	Ile	Gln	Leu	Phe	His	Lys	Lys	
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Ile	Glu	Ser	Ala	Leu	Arg	Asn	Lys	Met	Asn	Ser	Gln	Val	Cys	Glu	Lys	
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Val	Thr	Asn	Ser	Val	Ser	Ser	Lys	Leu	Gln	Pro	Tyr	Phe	Gln	Thr	Leu	
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- 5 -

Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys  
 210 215 220 225  
 Gly Glu Phe Tyr Ser Glu Asn His His Asn Pro Pro Pro Phe Ala Pro  
 230 235 240  
 Pro Val Met Glu Phe Pro Ala Ala His Asp Arg Met Val Tyr Leu Gly  
 245 250 255  
 Leu Ser Asp Tyr Phe Phe Asn Thr Ala Gly Leu Val Tyr Gln Glu Ala  
 260 265 270  
 Gly Val Leu Lys Met Thr Leu Arg Asp Asp Met Ile Pro Lys Glu Ser  
 275 280 285  
 Lys Phe Arg Leu Thr Thr Lys Phe Phe Gly Thr Phe Leu Pro Glu Val  
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 Ala Lys Lys Phe Pro Asn Met Lys Ile Gln Ile His Val Ser Ala Ser  
 310 315 320  
 Thr Pro Pro His Leu Ser Val Gln Pro Thr Gly Leu Thr Phe Tyr Pro  
 325 330 335  
 Ala Val Asp Val Gln Ala Phe Ala Val Leu Pro Asn Ser Ser Leu Ala  
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 Ser Leu Phe Leu Ile Gly Met His Thr Thr Gly Ser Met Glu Val Ser  
 355 360 365  
 Ala Glu Ser Asn Arg Leu Val Gly Glu Leu Lys Leu Asp Arg Leu Leu  
 370 375 380 385  
 Leu Glu Leu Lys His Ser Asn Ile Gly Pro Phe Pro Val Glu Leu Leu  
 390 395 400  
 Gln Asp Ile Met Asn Tyr Ile Val Pro Ile Leu Val Leu Pro Arg Val  
 405 410 415  
 Asn Glu Lys Leu Gln Lys Gly Phe Pro Leu Pro Thr Pro Ala Arg Val  
 420 425 430  
 Gln Leu Tyr Asn Val Val Leu Gln Pro His Gln Asn Phe Leu Leu Phe  
 435 440 445  
 Gly Ala Asp Val Val Tyr Lys  
 450 455